TriC to restore unfolded γ-crystallin to a native fold investigated using size exclusion chromatography. In collaboration with fellow members of the Center for Protein Folding Machinery, we are investigating the possibility of visualizing the crystallin substrate in the chaperonin substrate complex by Cryo-EM.

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2235-Pos Board B205
H2B-Crystallin Suppresses The Aggregation Upon Refolding Of Its Physiological Substrates H2D-, H2C- And H2S-Crystallin
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The passive chaperone α-crystallin, a small heat shock protein, is one of the ubiquitous crystallins in vertebrate lenses, along with the β-crystallins. We have studied the chaperone function of Human α2-crystallin interacting with its physiological Human γ-crystallin substrates. Human γD-crystallin (HγD-cryst) and γγ-crystallin (Hγγ-cryst) are stable and long-lived mammalian γ-crystallins localized in the lens nucleus. Human γS-crystallin (HγS-cryst) is abundant in the lens outer cortex. All three γ-crystallins can refold in vitro to their native state after unfolding in high concentrations of GdnHCl. However, at very low denaturant concentrations (1 M GdnHCl) aggregation of refolding HγC- and HγD-Crys intermediates competes with productive refolding. Diluting unfolded HγC-, HγD-, or HγS-Crys to low GdnHCl concentrations (100 µg/ml, 37°C) resulted in the protein population partitioning between productive refolding and aggregation pathways. HγD-, HγC- or HγS-Crys protein was allowed to refold and aggregate in the presence of H2B-Crys homo-oligomers at different monomer-to-monomer ratios of γ-Crys to βB-Crys. HγD- and Hγγ-Crys aggregation was suppressed to similar levels, whereas HγS-Crys aggregation was not suppressed as strongly in assays measuring solution turbidity at 350 nm. SEC chromatograms of the products of suppression reactions showed the presence of a high molecular weight complex containing the chaperone-substrate complex in ratios of 1γC:5γB and 1γD:5γB chains. This complex was still present 4 days after the suppression reaction was initiated. These results provide a model for how α-crystallin interacts with aggregation-prone substrates in vivo.

2236-Pos Board B206
Interaction between Molecular Chaperone Prefoldin with Group II Chaperonin in the Presence of Nucleotides: Implication for Substrate Transfer Mechanism from Prefoldin to Chaperonin
Tamotsu Zako1, Yosuke Murase1, Ryo Iizuka2, Taro Kanzaki3, Masafumi Shimizu1, Masafumi Yokoda1, Mizuo Maeda1.

Prefoldin (PFD) is a molecular chaperone that captures a protein-folding intermediate and transfers it to a group II chaperonin (CPN) for correct folding. However, mechanism of substrate transfer from PFD to CPN remains to be elucidated. Previous studies showed that CPN has a helical protrusion as a built-in-lid, and uses ATPase cycling to promote the conformational change necessary to open and close the lid. In this study, we have examined interaction between archael PFD and CPN in the presence of various nucleotide analogs. Affinities between fluorescein-labeled Pyrococcus PFD (PhPFD) and Thermococcus CPN (ThCPN) in the absence or presence of ADP and AMPPPN were examined by fluorescence anisotropy measurement. In the presence of ADP and AMPNNP, ThCPN was shown to take open and closed conformation, respectively. The affinity of PhPFD to ThCPN was weakest in the presence of AMPNNP, which suggests that PFD does not bind to CPN in closed-state. In contrast, PhPFFD bound more tightly to ThCPN (nucleotide free or ADP) in open-state. Interestingly, affinity of PhPFD to ADP-ThCPN was higher than nucleotide-free-ThCPN, even though both take open conformations. This result also implies that these open conformations are different, which is supported by other experiments indicating that ADP-ThCPN can suppress thermal aggregation more efficiently than nucleotide-free-ThCPN.

Our data implicates that substrate protein is delivered from PFD to CPN of the open conformation selectively in ADP-bound state rather than nucleotide-free-state.

2237-Pos Board B207
Networks of Functional Residues in GroEL and GroES
Riina Tehver, Jie Chen, D. Thurmalai.

The chaperonin GroEL and its cofactor GroES make up a molecular machine that rescues aggregation-prone misfolded proteins. The GroEL functional cycle consists of a series of large-scale allosteric transitions between the T, R, R’, and R” states. The corresponding large structural rearrangements facilitate substrate protein capture, refolding, and release, and are thus essential for the proper operation of the chaperonin. Using a Cα-sidechain elastic network model-based structural perturbation method, that probes the response of a local perturbation at all residue sites, we have studied the molecular details of the T → R and R” → T transitions and determined the key mechanical residues that support the allosteric cycle - the allosteric wiring diagram. We provide a molecular level interpretation for the intrarange positive cooperativity and interring negative cooperativity as well as the role of GroES in the GroEL allosteric cycle.

2238-Pos Board B208
ClpXP Degradation of the DNA-Protection Protein Dps Requires Auto-Tethering to the Enzyme
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Dps is a dodecameric bacterial protein that acts to prevent the formation of hydroxyl radicals and condenses cellular DNA to form “biochocats” under stressful conditions, protecting the chromosome from damage. During exponential growth, Dps is continually synthesized but rapidly degraded by the AAA+ protease ClpXP, resulting in a low cellular concentration. Dps degradation is rapidly turned off when cells respond to nutritional or oxidative stressors, allowing Dps to accumulate swiftly and counteract the damaging effects of the stressors. This environmental regulation of degradation is highly specific; stressors such as hydrogen peroxide result in the stabilization of Dps, while the degradation of other ClpXP substrates is not affected by this treatment. Maintenance of genomic integrity then crucially depends upon selective proteolysis of Dps by ClpXP only during non-stress conditions. The molecular mechanism of Dps recognition and degradation by ClpXP was probed through a combination of in vivo and in vitro techniques. Dps degradation exhibits an absolute requirement for the N-terminus of ClpX, a region that mediates interaction with substrate-delivery proteins called adaptors. The characterized ClpX adaptor SspB as well as a peptide representing only the ClpX-binding region of SspB are each able to compete efficiently with ClpXP for Dps degradation. The N-terminus of Dps seems to interact with ClpX, primarily on its N-domain. An extended region or multiple regions within the N-terminus of Dps are required for efficient competition of Dps degradation by ClpXP. Thus, Dps functions analogously to an adaptor protein by using its unstructured N-terminus to tether itself to ClpX during the degradation process. This mechanism may increase the affinity of Dps for ClpX by allowing the two proteins to engage in multiple contacts simultaneously.
we find that Hsp90 mutants deficient for ATP binding or hydrolysis have differential impacts on the activation of kinase and hormone receptor clients in vivo. These results provide a rationale for understanding anti-cancer drugs that competitively bind to the ATPase site of Hsp90.

Heme Proteins

2240-Pos Board B210 Reactive Vibrational Dynamics of Iron in Heme Alexander Barabanschikov1, J. Timothy Sage1, W. Robert Scheidt2, Chuanjiang Hu2, Minoru Kubo3, Paul M. Champion1, Jiyong Zhao4, Wolfgang Sturhahn5, E. Erkan Alp6
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Nuclear resonance vibrational spectroscopy (NRVS) measurements supported by DFT calculations identify vibrational modes of the iron atom in halide derivatives of iron porphyrins. These compounds capture many essential aspects of heme geometry and vibrations. The smaller (porphine) models simplify the vibrational spectrum and enable accurate analysis using DFT methods. NRVS identifies both doming and Fe-halide stretching components of the reaction coordinate with confidence. Correlation analysis between 4-coordinate and 5-coordinate compounds suggests significant mixing between Fe-ligand and heme modes. Measurements and calculations on larger porphyrins reveal the effect of peripheral groups. Measurements on oriented porphine halide crystals definitively identify the contribution of in plane and out of plane Fe motion. The frequency of heme doming vibrations varies in a systematic manner with peptide substitute and halide mass, which will allow us to evaluate their contributions to vibrational signals that follow femtosecond laser excitation. Such measurements will ultimately enable quantitative estimates of the energetics of molecular distortions that modulate reaction rates in heme proteins.

2241-Pos Board B211 Low frequency dynamics of Cystathionine beta-synthase Karunakaran Venugopal1, Yuhua Sun2, Zhengu Zhang1, Abdulkrim Benabbs1, Sangita Singh1, Ruma Banerjee2, Paul M. Champion1
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Femtosecond coherence spectroscopy is used to study the low frequency dynamics of cystathionine beta-synthase (CBS). CBS is a pyridoxal-5'-phosphate-dependent heme enzyme with cysteine and histidine axial ligands that catalyzes the condensation of serine and homocysteine to form cystathionine.

2242-Pos Board B212 Proximal Ligand Switch Triggered by Carbon Monoxide in Inducible Nitric Oxide Synthase Joseph Sabat, Denis L. Rousseau, Syun-Ru Yeh
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Inducible Nitric Oxide Synthase (iNOS) is one of three isoforms of NOS, responsible for the oxidation of L-Arginine to L-citrulline and nitric oxide (NO). The iNOS isoform is implicated in the pathophysiology of several inflammatory disorders including arthritids, atherosclerosis, and transplant rejection. iNOS is unique among the isoforms in that it is not regulated by the intracellular calcium concentration. Instead, iNOS is exclusively regulated at the transcriptional and molecular levels. One molecular regulator of iNOS is Carbon Monoxide (CO) generated by heme oxygenase (HO-1), an inducible enzyme that produces CO and is known to mediate anti-inflammatory effects. It has been shown that CO binding to iNOS promotes its gradual conversion to an inactive "p420" form. On this basis, we hypothesize that the cross-talk between HO-1 and iNOS plays an important role in attenuating the activity of iNOS and modulating inflammatory responses in vivo. The structure of the iNOS p420 has been a subject of debate, as the proximal ligand has been proposed to be either a histidine residue or a protonated, neutral thiol form of the native cysteine thiolate. In this work, we use resonance Raman Spectroscopy to explore the properties of the p420 derivative of iNOS in order to resolve this issue. We show that the nanosecond time-resolved Raman spectrum of iNOS p420 exhibits a band consistent with an iron-histidine stretching mode. To evaluate the identity of the proximal ligand of iNOS p420, we measured the Raman spectra of the H93G cavity mutant and organic model compounds with a neutral thiol coordinated to them as reference systems. On the basis of these studies, we postulate a novel reversible ligand-switching mechanism that may be critical for the in vivo regulation of iNOS activity involving endogenous CO.

2243-Pos Board B213 Resonance Raman Investigation of the R481 Mutants of Cytochrome c Oxidase from R. sphaeroides Tsyoshi Egawa1, Hyun-Ju Lee2, Robert B. Gennis2, Syun-Ru Yeh1, Denis L. Rousseau1
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The enzymes of the heme-copper oxidase superfamily have a highly conserved arginine residue (R438, R481, and R473 of the bovine, R. sphaeroides and P. denitrificans cytochrome c oxidases, respectively), which is located close to the copper-oxygen atom and propionate substituents of the heme a and heme a3 moieties. To explore the structural and functional implications of this conserved arginine, we used resonance Raman spectroscopy to study the heme vibrational spectra of the R481 mutant proteins (R481H, R481Q, and R481L) of cytochrome c oxidase from R. sphaeroides. All the mutants showed significant down-shifts in the C=O stretching vibrational frequencies of the heme ap and a3 formyl substituent groups in the fully oxidized state, while they showed up- and down-shifts of the a3 and a3 formyl C=O stretching modes, respectively, in the fully reduced state. On the basis of these observations, the role of the conserved arginine will be discussed.

2244-Pos Board B214 Indoleamine 2, 3-Dioxygenases 2: The Missing Link For The 1-methyl-D-trp Mechanism Of Action? Laura B. Granell-Ortiz, Syun-Ru Yeh
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Indoleamine 2, 3-dioxygenase (IDO1) is one of the only two heme-containing enzymes that catalyze the first and rate-limiting step of the kynurenine pathway of L-Trp metabolism. IDO1 has been implicated in the escape mechanism of cancer cells from immune surveillance. Consequently, IDO1 has been recognized as an important immune suppressor drug target. Recent studies showed that an IDO1 inhibitor, 1-methyl-Trp (1-M-Trp), triggers antitumor immunity and can be used to improve the efficacy of traditional chemotherapeutic drugs. Preclinical studies showed that the D stereoisomer of 1-M-Trp exhibits superior antitumor activity; however, it is less potent for the purified enzyme. On this basis, a second isoform of IDO1 had been suspected. It was not until last year that this second isoform of IDO1, named IDO2, was identified. To understand the missing link for the D-1-M-Trp mechanism of action, we have cloned, expressed and purified recombinant human IDO2. Resonance Raman and optical absorption spectroscopic studies showed that IDO2 exhibits structural features slightly different from IDO1. Furthermore, an activity assay with 1-M-Trp demonstrated as an important anticancer drug target. Based on these observations, the role of the conserved arginine will be discussed.

2245-Pos Board B215 Linking Heme Activation to Conformation Change in Hemoglobin Via Chain Selective Time-resolved Resonance Raman Spectroscopy on Meso-heme Hybrids Gurusamy Balakrishnan1, Mohammed Ibrahim1, Piotr J. Mak2, Jessica Hata2, James R. Kincaid2, Thomas G. Spiro1
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Time-resolved Resonance Raman spectra are reported for Hb tetramers, in which the β and β chains are selectively substituted with mesoheme. Hb function is unaffected by the substitution, but the Soret absorption band shift in meso-related to proto-heme permits chain-selective excitation of heme RR spectrum. The evolution of these spectra following hBco photolysis show that gametate